Biomolecules & Therapeutics, **18(4)**, 411-419 (2010)
www.biomolther.org

ISSN: 1976-9148(print)/2005-4483(online)
DOI: 10.4062/biomolther.2010.18.4.411

Effect of Korean Mistletoe Lectin on Gene Expression Profile in Human T Lymphocytes: A Microarray Study

Su-Yun LYU, and Won-Bong PARK[1,*]

Department of Herbal Medicinal Pharmacology, Daegu Haany University, Gyeongsan 712-715,
[1]Department of Chemistry, Seoul Women's University, Seoul 139-774, Republic of Korea

(Received July 9, 2010; Revised August 4, 2010; Accepted August 4, 2010)

Abstract − Korean mistletoe has a variety of biological effects, such as immunoadjuvant activities. This study investigates the effects of Korean mistletoe lectin (*Viscum album* L. var. *coloratum* agglutinin, VCA) on human T lymphocytes to determine whether VCA acts as an immunomodulator. Purified human T-lymphocytes were cultured with VCA and RNA from each point was analyzed using Affymetrix human genome chips containing 22,500 probe sets which represents more than 18,000 transcripts derived from 14,500 human genes. As a result, there was a striking upregulation of genes coding for chemokines. Seventeen genes out of 50 coding for proteins with chemokine activity were upregulated including CXCL9 and IL-8 which are related to the treatment of cancer. In addition, 28 cytokine genes were upregulated including IL-1, IL-6, IL-8, IFN-γ, and TNF-α. Taken together, the data suggest that Korean mistletoe lectin, in parallel with European mistletoe, has an ability to modulate human T cell function.

Keywords: DNA microarray, Korean mistletoe, Lectin, Gene expression, T lymphocytes

INTRODUCTION

Mistletoe belongs to the family of *Loranthaceae* and *Viscaceae*, which are taxonomically related to each other (Barlow, 1983). Application of *Viscum album* L. is one of the most widely used unconventional remedies in Europe to treat hypertension, arteriosclerosis, diabetes, arthritis, and even more importantly, cancer (Büssing, 2000). The most prominent effect of *Viscum album* extract (VA-E) is their cytotoxic property (Büssing, 2000). There are growing evidence that mistletoe extract can induce apoptotic killing of cultured human tumor cells and lymphocytes (Hajto *et al.,* 1990; Schultze *et al.,* 1991; Park *et al.,* 2001; Urech *et al.,* 2005). Furthermore, European mistletoe lectin (*Viscum album* L. agglutinin, VAA) was found to modulate cells including human lymphocytes, monocytes, neutrophils, and cancer cells (Hostanska *et al.,* 1996; Savoie *et al.,* 2000; Lavastre *et al.,* 2002).

Asian mistletoe which is distributed in Korea and other East-Asian countries (mainly in China and Japan) has also been recognized as a therapeutic herb (Li, 1975). Among its components, a galactose- and *N*-acetyl-D-galactosamine-specific lectin (*Viscum album* L. var. *coloratum* agglutinin, VCA), which is known for its anti-cancer activity, was isolated from Korean mistletoe using SP Sephadex-C50, Sepharose 4B, and ultrafiltration (Lyu *et al.,* 2000; Lyu *et al.,* 2001; Lyu *et al.,* 2002). Although there is less research conducted for Korean mistletoe compared to the European's, there are increasing evidence that Korean mistletoe possesses similar cytotoxic and immunological activities compared to the European's (Yoon *et al.,* 1999; Lyu *et al.,* 2000; Lyu *et al.,* 2001; Pae *et al.,* 2001; Park *et al.,* 2001; Yoon *et al.,* 2003; Lyu and Park, 2007).

Microarray analysis allows simultaneous monitoring of expression of multiple genes in different cells and tissues. Its applications for pharmaceutical and clinical research include identification of disease-related genes, as well as targets for therapeutic drugs (Alizadeh *et al.,* 2000). In this study, we were interested in obtaining proof of principle data in human T-cells by analyzing the gene expression after VCA treatment using microarray techniques. Affymetrix Human Genome U144 Plus 2.0 Array containing more than 54,000 probe sets representing greater than 47,400 transcripts derived from approximately 39,000 well-substantiated human genes was used. We then used bio-

*Corresponding author
Tel: +82-2-970-5655 Fax: +82-2-975-3159
E-mail: wbpark@swu.ac.kr

informatics tools to analyze the differentially-regulated genes in order to identify common transcription regulators. Using this approach, we identified a genetic regulatory network that responds to VCA.

MATERIALS AND METHODS

Isolation of lectin

Korean mistletoe growing on oak trees was collected in Kangwong province, Korea, during winter. The botanical identity was established by Prof. Jon Suk Lee, College of Natural sciences, Seoul Women's University. Crude protein was prepared by ion exchange chromatography and affinity chromatography as described previously (Park *et al.,* 1998a).

Gel electrophoresis

The molecular mass and purity of VCA was determined by sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE). Twelve percent polyacrylamide (Sigma, Poole, UK) was used as the 'resolving gel' and 4% acrylamide was used as the 'stacking gel'. The resolving and stacking gel were polymerized for 30 and 10 min, respectively. To further denature the protein by reducing disulfide linkages, samples were heated at 98°C for 10 min in the presence of 1% 2-mercaptoethanol (Sigma). Bromophenol blue (0.1 mg/ml) was also added to the protein solution to serve as a tracking dye and the samples were loaded into the wells. The samples were electrophoresed using the Bio-Rad electrophoresis system (CA, USA) at 100 V for 1 hr. The gel was stained with Coomassie Brilliant Blue R-250 (Sigma) for 1 hr and was destained for protein determination.

Purification and evaluation of human T-lymphocytes

To prepare peripheral blood mononuclear cells (PBMC), human peripheral blood (30 ml) was obtained after the permission of healthy human volunteers (age ranging from 25 to 40 years) with the addition of 100 unit/ml heparin. PBMC was isolated by the Histopaque gradient density method according to the manufacturer's instructions (Sigma). Human T-lymphocytes were isolated from human PBMC using MACS Pan T-lymphocyte Isolation Kit II (Miltenyi Biotec Inc., CA, USA). T-lymphocyte purity was evaluated via FACS analysis using a fluorescence- activated cell sorter (FACSCalibur; BD Biosciences, Oxford, UK).

Cell culture

Human PBMC and T-lymphocytes were maintained in a complete tissue culture medium (CTCM) consisting of RPMI 1640 (Sigma) supplemented with 5% fetal calf serum (FCS, Sigma), 100 U/ml penicillin/100 μg/ml streptomycin (Sigma), 2 mM -glutamine (Sigma), and 5×10^{-5} M 2-mercaptoethanol (2-ME, Sigma). Cell numbers and viability were assessed by the trypan blue (Sigma) dye exclusion method.

Viability test

The *in vitro* cell viability was measured using solutions of a novel tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethyoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS, Promega, Southampton, UK) and an electron coupling reagent (phenozine methosulphate; PMS, Promega). The production of formazan was determined by measuring the absorbance of the compound at 450nm with a spectrophotometric 96-well plate reader (Dynex Technologies, VA, USA).

Preparation for DNA microarray analysis

Total cellular RNA was extracted from cells using RNeasy® Mini kit (Qiagen, Valencia, CA, USA). The concentration was determined by NanoDrop® ND-1000A UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Also, the quality of RNA was checked with an Agilent 2100 Bioanalyzer Analysis System (Agilent Technologies, Palo Alto, CA, USA) before performing the microchip arrays. First and second strand cDNA were synthesized from total RNA using the 'GeneChip® One-Cycle Target Labeling and Control Reagents Kit' which contains 1 IVT (*In Vitro* Transcription) Labeling Kit, 1 One-Cycle cDNA Synthesis Kit, 1 Sample Cleanup Module, 1 Poly-A RNA Control Kit, and 1 Hybridization Control Kit (Affymetrix, Santa Clara, CA, USA). After cooling the sample at 4°C, 130 μl of Second-Strand Master Mix (91 μl of RNase-free water, 30 μl of 5×2^{nd} Strand Reaction Mix, 3 μl of 10 mM dNTP, 1 μl of *E. coli* DNA ligase, 4 μl of *E.coli* DNA Polymerase I, and 1 μl of RNase H) was added to the first-strand synthesis sample. For IVT (*In Vitro* Transcription) reaction, 4 μl of 10×IVT Labeling Buffer, 12 μl of IVT Labeling NTP Mix, and 4 μl of Labeling Enzyme Mix were added to the sample, and were filled up with RNase-free water to give a final reaction volume of 40 μl. The mix was incubated at 37°C for 16 hr. Sixty microliters of RNase-free water, 350 μl of IVT cRNA Binding Buffer, and 250 μl of ethanol (99.5%) were added to the IVT reaction, and the sample was applied to the IVT cRNA Cleanup Spin Column. cRNA yield was determined using the NanoDrop® ND-1000A UV-Vis Spectrophotometer (NanoDrop Technologies) as described above. The size distribution of cRNA was evaluated using the Agilent 2100 Bioanalyzer Analysis

System (Agilent Technologies).

Hybridization and probe array scanning

Human Genome U133 Plus 2.0 Array (Affymetrix) which contains more than 54,000 probe sets representing greater than 47,400 transcripts derived from approximately 39,000 well-substantiated human genes was used for hybridization. The hybridization cocktail for the complete Human Genome U133 Plus 2.0 Array was prepared by mixing 15 μg of fragmented cRNA, 5 μl of 3 nM Control Oligonucleotide B2, 15 μl of 20×Eukaryotic Hybridization Controls (*bioB*, *bioC*, *bioD*, *cre*), 3 μl of 10 mg/ml Herring Sperm DNA, 3 μl of 50 mg/ml BSA, 150 μl of 2× Hybridization Buffer, and 30 μl of DMSO. The probe array was placed into the Hybridization Oven and was rotated at 60 rpm at 45°C for 16 hr. To wash, stain, and scan a probe array, the Fluidics Station 450/250 and the GeneChip® Operating Software (GCOS) (Affymetrix) were used.

Statistical data analysis for microarray

The scanned images were analyzed using the Affymetrix Microarray software. Sample loading and variations in staining were standardized by scaling the average of the fluorescent intensities of all genes on an array to constant target intensity (250) for all arrays used. Data analysis was conducted using the GeneChip® Operating Software (GCOS, Affymetrix) and the Affymetrix® NetAffx™ Analysis Center (www.affymetrix.com/analysis).

RESULTS

Purification of VCA

VCA was isolated from Korean mistletoe using the affinity chromatography, SP Sephadex C-50, and asialofetuin-Sepharose 4B as previously described (Park *et al.,* 1997; Park *et al.,* 1998b). In the absence of the reducing agent (2B-mercaptoethanol), the molecular mass of VCA was 60 kDa, which is similar to the molecular mass of European mistletoe lectin (VAA). In the presence of the reducing agent, VCA showed two bands consisting of a 30 kDa A-chain and a 34 kDa B-chain, whereas European mistletoe lectin showed three bands (36 kDa, 33 kD, and 29 kDa) (Fig. 1). Compared with results previously reported (Park *et al.,* 1997; Park *et al.,* 1998b), lectin was efficiently isolated by the asialofetuin-immobilized chromatography.

Purity evaluation of human T-cells using FACS

The purity of the enriched T-cells was evaluated by flow cytometry. The cell fraction was stained with fluorescently labeled antibodies (CD25-PE 5 μg/tube to detect T cell activation and CD3-ECD 5 μg/tube to detect T cell purity) and the percentage of the populations was evaluated by FACS analysis. Cell debris and dead cells were excluded from

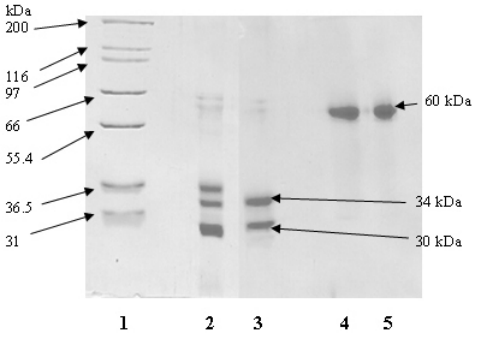

Fig. 1. SDS-PAGE profiles of VCA. Korean (VCA) and European mistletoe lectin (VAA) were analyzed by SDS-PAGE. (A) Crude proteins were prepared by the ion exchange chromatography and SP Sephadex C-50, in the presence (lane 2, 3) and absence (lane 4, 5) of a reducing agent. Lane 1; molecular weight marker, lane 2, 4; European mistletoe proteins, lane 3, 5; Korean mistletoe proteins.

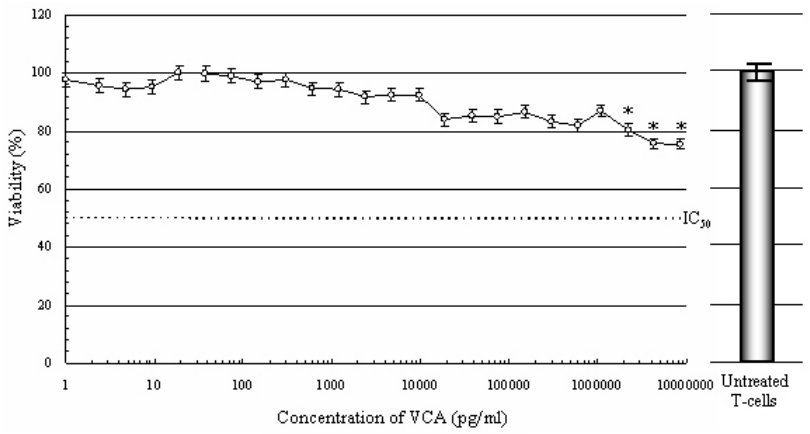

Fig. 2. A composite of three independent cell viability tests performed on human T-cell. T-cells were cultured in the absence or presence of different concentrations of VCA and the viability was measured by MTS assay. Student's t-test was used to compare each of the treatment groups with the controls and the significance was determined. *$p < 0.05$.

the analysis based on scatter signals. As a result, a purity of 92% of T cells could be achieved.

Effect of VCA on T cell viability

In order to verify that the administration dose had minimal effect on the cells, the viability of human T-lymphocytes was measured. Cells were treated for 48 hours with VCA at the indicated dose (1 pg/ml up to 1.6 μg/ml) and were measured using the MTS assay. As a result, T-lymphocytes showed 80% viability when they were treated with 800 ± 10 ng/ml of VCA (Fig. 2). The result obtained showed that the selected concentrations of VCA (10 pg/ml, 600 pg/ml, and 1.5 ng/ml) for the microarray analysis had no major effect on the cells when compared to the controls.

Overall changes in gene expression profiles of T cells by VCA

To study the effects of Korean mistletoe lectin, VCA was applied at the following concentrations to determine the immunological activity: 10 pg/ml VCA, 600 pg/ml VCA, and 1.5 ng/ml VCA, for varied time intervals (2 and 8 hr) on human T-cells. Each gene chip in the HG-U133 Plus 2.0 GeneChip™ Array set represented approximately 39,000 genes and expressed sequence tags. The cluster analysis achieved shows the overall expression pattern and the biological correlation of replicates in cellular response to VCA treatment over several time points and concentrations (Fig. 3). To obtain a global view of the differential gene expression, genes that were either consistently increased or decreased in all sets of experiment with respect to time intervals and concentrations were focused on. All probe sets designated 'absent' by the GeneSpring software were re-

	Resting Cell	VCA 10 pg/ml	VCA 600 pg/ml	VCA 1.5 ng/ml
2 hr	Group (1)	Group (4)	Group (3)	Group (2)
8 hr	Group (5)	Group (8)	Group (7)	Group (6)

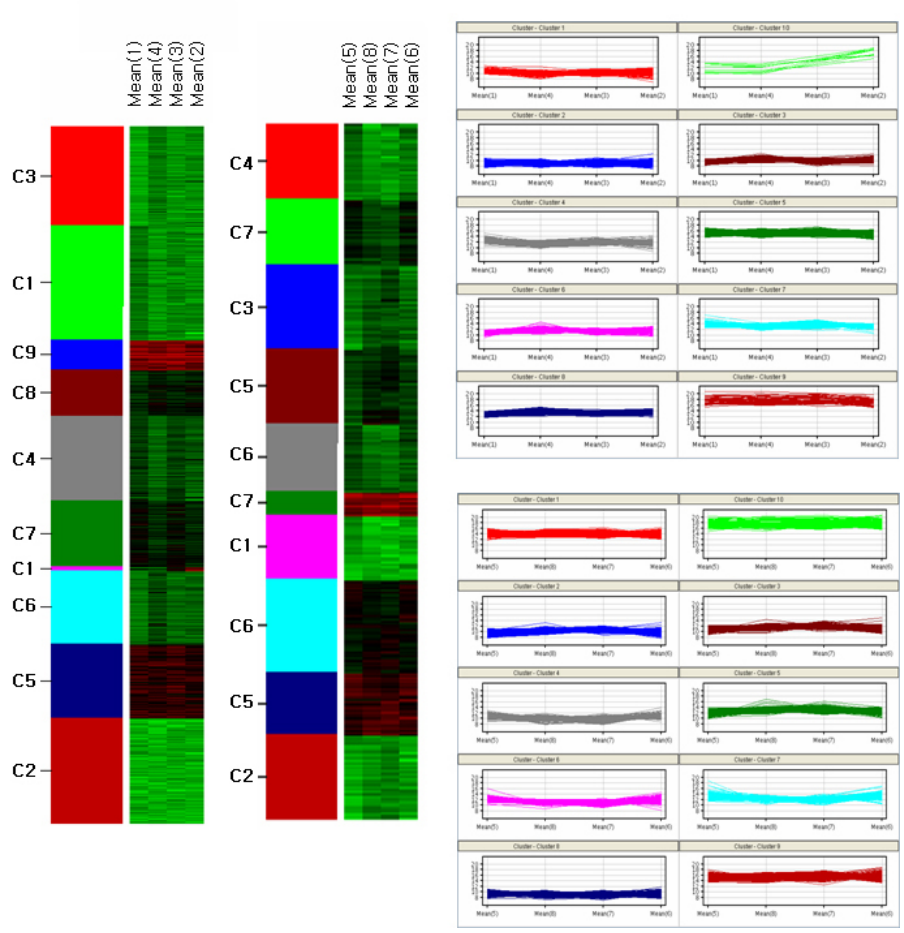

Fig. 3. Clustering analysis. The cluster analysis shows the overall expression pattern and the biological correlation of replicates in cellular response to VCA treatment over several time points and concentrations. Ten clusters from 4156 genes were achieved from Volcano plot (cutoff 2.0, Welch's T-test significance 0.05), clustering (Gene clustering and sample clustering), hierarchical clustering (with Ward's linkage), and similarity measure using Euclidean distance.

moved, and only those genes whose expressions changed by 2-fold or greater in at least two pairwise comparisons were taken as significant. Using a cutoff value of ≥ ± 2-fold change in transcript abundance in all experiments, a total of over 3500 genes were identified as relatively responsive to VCA. VCA responsive genes were divided into functional classes based on gene ontology (http://www.go-database/org/cgi-bin/go.cgi) including biological function, cellular component, and molecular function in all sets of experiment.

Gene modulation of the chemokine family by VCA

The production of chemokines is induced by exogenous irritants and endogenous mediators such as interleukin (IL)-1, tumor necrosis factor-alpha (TNF-α), platelet-derived growth factor (PDGF), and interferon-gamma (IFN-γ). In this study, the most striking family of genes modulated by VCA is the chemokine family. Seventeen genes, out of 50 coding for proteins with chemokine activity on the array, were upregulated. These genes code for chemokines of the C-C (CCL2, CCL3, CCL4, CCL8, CCL13, CCL14, CCL15, CCL17, CCL18, CCL20, CCL24) and CXCL families (CXCL1, CXCL2, CXCL3, CXCL5, CXCL9, CXCL13), and also for IL-8 (Table I and II).

Gene modulation of cytokines by VCA

In the present study, VCA was found to stimulate, at non-toxic variable concentrations (10 pg/ml, 600 pg/ml, and 1.5 ng/ml), the gene expression of the cytokines including IL-1α, IL-1β, IL-6, IL-8, IFN-γ, and TNF. The amounts of cytokines tested with VCA were high, particularly for IL-1α and IL-1β, and to a lesser extent for TNF-α (Table I and II). The lesser secretion of TNF-α might be related to a passive release of cytokines following the secretion of IL-1. These results are consistent with the previous reports on the secretion of cytokines (Hajto *et al.,* 1990; Männel *et al.,* 1991; Hostanska *et al.,* 1995; Riberéau-Gayon *et al.,* 1996; Möckel *et al.,* 1997; Riberéau-Gayon *et*

Table I. Genes modulated by VCA for 2 hr with various concentrations. Genes with immunological significance (>2 fold-change) are shown

UniGene ID	Gene description	Gene symbol	VCA 10 pg/ml	VCA 600 pg/ml	VCA 1.5 ng/ml
Chemokine					
hCG27669.2	CCL18	Chemokine (C-C motif) ligand 18	1.86	3.32	3.47
hCG16364.3	CXCL5	Chemokine (C-X-C motif) ligand 5	1.99	5.06	3.30
hCG14841.3	CCL20	Chemokine (C-C motif) ligand 20	1.25	1.54	2.48
hCG22991.2	CXCL13	Chemokine (C-X-C motif) ligand	−3.62	2.22	2.24
hCG16361.2	CXCL2	Chemokine (C-X-C motif) ligand 2	1.16	1.32	2.17
hCG16366.3	CXCL3	Chemokine (C-X-C motif) ligand 3	1.08	1.42	2.12
hCG16372.3	IL8	Interleukin 8	−1.17	1.23	2.03
hCG29301.2	CCL8	Chemokine (C-C motif) ligand 8	−2.78	1.35	1.01
hCG32604.3	CCL15;CCL14	Chemokine (C-C motif) ligand 14	−2.17	−1.08	−1.12
hCG1647381.3	CCL24	Chemokine (C-C motif) ligand 24	−2.20	2.19	−1.28
hCG16481.3	CCL17	Chemokine (C-C motif) ligand 17	−2.97	−1.73	−2.29
Cytokine					
hCG1741698.1	IFNA1	Interferon, alpha 1	2.26	2.51	4.24
hCG23042.3	TNFSF9	Tumor necrosis factor (ligand) superfamily	1.25	1.36	3.37
hCG17267.3	INHBA	Inhibin, beta A	1.35	2.68	2.03
hCG15987.3	IFNG	Interferon, gamma	2.81	2.66	1.93
hCG33094.2	TNFSF5	Tumor necrosis factor (ligand) superfamily	2.01	1.29	1.11
hCG1991317	TNFSF12	Tumor necrosis factor (ligand) superfamily	−2.61	1.13	−1.08
hCG1733963.2	IL1RN	Interleukin 1 receptor antagonist	−2.13	−1.03	−1.22
Growth factor					
hCG45297.2	DTR	Diphtheria toxin receptor	1.53	1.85	2.14
hCG2020168	FLT1	Fms-related tyrosine kinase 1	2.33	1.81	1.43
hCG14780.3	NRG1	Neuregulin 1	−1.51	−1.16	−2.01
Others					
hCG2025762	BOMB	BH3-only member B protein	−1.28	1.64	2.03
hCG16948.3	HUMGT198A	GT198, complete ORF	2.00	1.10	1.84
hCG1759019.2	HOMER1	Homer homolog 1	2.22	1.04	1.75
hCG41839.3	LGALS2	Lectin, galactoside-binding, soluble, 2	−2.38	1.29	1.03
hCG28281.3	TRAF4	TNF receptor-associated factor 4	−1.55	−2.03	−1.08
hCG1785346.3	PRKCBP1	Protein kinase C binding protein 1	−3.58	−1.32	−1.75
hCG25928.2	MLF2	Myeloid leukemia factor 2	−2.39	−1.24	−1.88
hCG2014701	LRRC10	Leucine rich repeat containing 10	−1.14	−1.48	−2.32

Table II. Genes modulated by VCA for 8 hr with various concentrations. Genes with immunological significance (> 2 fold-change) are shown

UniGene ID	Gene description	Gene symbol	VCA 10 pg/ml	VCA 600 pg/ml	VCA 1.5 ng/ml
Chemokine					
hCG16372.3	IL8	Interleukin 8	1.28	4.88	18.18
hCG32604.3	CCL15; CCL14	Chemokine (C-C motif) ligand 14	−1.49	1.53	16.65
hCG16366.3	CXCL3	Chemokine (C-X-C motif) ligand 3	−1.05	2.71	15.70
hCG1749202.1	CCL3	Chemokine (C-C motif) ligand 3-like	−2.03	2.45	14.53
hCG16368.2	CXCL1	Chemokine (C-X-C motif) ligand 1	1.55	3.54	8.15
hCG29301.2	CCL8	Chemokine (C-C motif) ligand 8	3.15	35.19	7.77
hCG16361.2	CXCL2	Chemokine (C-X-C motif) ligand 2	−1.50	1.03	7.71
hCG16364.3	CXCL5	Chemokine (C-X-C motif) ligand 5	5.56	9.74	7.34
hCG1748235.2	CCL4L	Chemokine (C-C motif) ligand 4-like	−2.00	1.05	5.24
hCG14841.3	CCL20	Chemokine (C-C motif) ligand 20	−1.33	−1.08	5.06
hCG29298.3	CCL2	Chemokine (C-C motif) ligand 2	1.73	3.70	3.91
hCG16481.3	CCL17	Chemokine (C-C motif) ligand 17	1.81	2.70	1.33
hCG22991.2	CXCL13	Chemokine (C-X-C motif) ligand 13	2.18	2.21	1.24
hCG1647381.3	CCL24	Chemokine (C-C motif) ligand 24	2.10	3.36	1.22
hCG29297.1	CCL13	Chemokine (C-C motif) ligand 13	2.16	9.91	1.18
hCG27669.2	CCL18	Chemokine (C-C motif) ligand 18	2.97	4.05	−1.27
Cytokine					
hCG16260.3	IL1A	Interleukin 1, alpha	−1.51	3.95	48.27
hCG17267.3	INHBA	Inhibin, beta A	−1.10	10.82	37.07
hCG16263.2	IL1B	Interleukin 1, beta	−2.40	2.53	21.70
hCG38231.4	IL6	Interleukin 6 (interferon, beta 2)	1.01	4.57	17.70
hCG15987.3	IFNG	Interferon, gamma	−4.05	−1.75	4.46
hCG1733963.2	IL1RN	Interleukin 1 receptor antagonist	1.79	10.77	4.05
hCG27245.2	IL1F6	Interleukin 1 family, member 6	2.30	1.26	1.44
hCG20249.3	TNFSF10	Tumor necrosis factor (ligand) superfamily	−1.01	2.29	1.13
hCG33094.2	TNFSF5	Tumor necrosis factor (ligand) superfamily	−1.26	−2.96	−1.07
hCG1741698.1	IFNA1	Interferon, alpha 1	−1.19	−2.83	−1.13
hCG28387.3	TNFSF13B	Tumor necrosis factor (ligand) superfamily	1.59	3.28	−1.57
Growth factor					
hCG45297.2	DTR	Diphtheria toxin receptor	−1.68	1.60	5.00
hCG24963.2	FGF6	Fibroblast growth factor 6	2.42	1.40	1.79
hCG2001992	MST1	Macrophage stimulating 1	−7.61	−22.70	−1.22
hCG40536.2	PDGFD	Platelet derived growth factor D	−2.69	−2.59	−1.39
hCG14780.3	NRG1	Neuregulin 1	−1.24	−1.43	−2.30
Others					
hCG27634.3	TRAF2	TNF receptor-associated factor 2	1.60	2.18	3.13
hCG30334.3	TRAF1	TNF receptor-associated factor 1	−1.38	1.11	2.78
hCG41839.3	LGALS2	Lectin, galactoside-binding, soluble, 2	−2.01	1.13	−3.08

al., 1997).

Other genes modulated by VCA

Among the Fc receptors, the gene coding for FCAR (Fc fragment of IgA receptor) was increased whereas genes coding for FCER1A (Fc fragment of IgE receptor, high affinity I), FCER1G (Fc fragment of IgE receptor, high affinity I, gamma polypeptide), FCGR2C (Fc fragment of IgG receptor, low affinity IIc (CD32)), and FCGRT (Fc fragment of IgG receptor, transporter, alpha) were decreased. Among growth factors, FGF6 (fibroblast growth factor 6), FLT1 (fms-related tyrosine kinase 1), and DTR (diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)) were increased, and MST1 (macrophage stimulating 1 (hepatocyte growth factor-like)), NRG1 (neuregulin 1), and PDGFD (platelet derived growth factor D) were decreased (Table I and II).

DISCUSSION

The beneficial effects of mistletoe preparations and some of their components have been demonstrated in several *in vitro* and *in vivo* studies (Büssing, 1997). However, their effects on gene expression in human immune cells have rarely been studied. Modulation of the immune response may be manifested in various ways. Immunosuppression may lead to an enhanced host susceptibility to infectious or neoplastic disease, whereas immunostimulation (immunoenhancement) may ultimately result in hyperimmune conditions including allergy or autoimmune dis-

ease (Gleichmann *et al.,* 1989). Therefore, we used microarray analysis to examine the effects of VCA on human T lymphocytes. As a result, we assessed the level of over 39,000 genes in VCA-treated human T cells and observed marked alterations in the expression of 17,393 genes. These genes are involved in immune and defense responses, signal transduction, DNA modification and replication, regulation of transcription, and transport in a wide range of biological systems.

Most importantly, there was a striking upregulation of genes coding for chemokines. Chemokines are crucial during an inflammatory response for a timely recruitment of specific leukocyte subpopulations to sites of tissue damage. In addition, they are also important in noninflammatory functions such as dendritic cell maturation, B, and T cell development, Th1 and Th2 responses, infections, angiogenesis, tumor growth, and metastasis (Rossi and Zlotnik, 2000). Seven genes out of twenty one were upregulated more than 10-fold by VCA code for chemokines. In particular, VCA induced the upregulation of C-C and CXCL families (including CXCL9), and also for IL-8. An analysis of the cellular distribution of CXCR3 (Homey *et al.,* 2002), the receptor for CXCL9, suggests that VCA is likely to activate Th1, CTL, and NK cells. CXCL9 possess strong angiostatic properties that make them interesting drugs for the treatment of cancer (Rossi and Zlotnik, 2000). Thus, this finding lends support to the therapeutic potential of VCA in the treatment of cancer. IL-8, a member of the CXC chemokine family, is a prototypical chemokine and its most remarkable property is the wide variation in its level of expression in response to cellular stress. IL-8 is known to be produced by monocytes and macrophages, and its secretion is induced by a number of stimuli, including pro-inflammatory cytokines ('Stvrtinová *et al.,* 1995). Therefore the upregulation of IL-8 may be the result of several pro-inflammatory cytokines increment, such as IL-1β and TNF-α.

In contrast to chemokines, cytokine modulations by mistletoe have been discussed intensively (Hajto *et al.,* 1990; Joshi *et al.,* 1991; Männel *et al.,* 1991; Hostanska *et al.,* 1995; Hostanska *et al.,* 1996; Möckel *et al.,* 1997; Riberéau-Gayon *et al.,* 1997; Yoon *et al.,* 2001). Thirty three genes on the array that code for proteins with cytokine activity were identified. Twenty eight cytokine genes were upregulated by VCA (including IL-1α, IL-1β, IL-6, IL-8, IFN-γ, and TNF-α), and five of the genes were upregulated more than 10-fold. The above observations are consistent with the previous studies (Frantz, 1989; Beuth *et al.,* 1994; Riberéau-Gayon *et al.,* 1996) which showed that low non-toxic concentrations of mistletoe lectin can stimulate animal or human macrophages/monocytes.

Taken together, these data suggest that a specific network of chemokine genes is activated by VCA, and that this activation is accompanied by a similar activation of the cytokine network. The expression of chemokines is triggered by several cytokines, such as IL-1β and TNF-α, through a nuclear factor kappa B (NF-κB)-mediated event; by IFN-γ through the Janus-activated kinase (JAK)-signal transducer and activation of transcription (STAT) pathway; or by activated protein 1 (AP-1)-mediated transcription. The transcription of chemokine genes is also often inhibited by the transforming growth factor (TGF)-β (Richmond, 2002). Indeed when human T cells were stimulated with VCA, genes associated with pathways regulating chemokine production were found in this data, including IL-1β, NF-κB, STAT (1, 2, 4, 6), and TGF-β.

In agreement with the pleiotypic biological properties of VCA, the numbers of mechanisms that can be discussed based on these data are large. Fc- and complement receptors are major targets of immunoglobulins (Heyman, 1996; Reilly and McKenzie, 2002). Among the Fc receptors, the gene coding for FCAR is increased whereas the gene coding for FCER1A, FCER1G, FCGR2C, and FCGRT are decreased. This differential expression suggests that these genes may be a suitable target for the biological activity of VCA in human T cells. Among the complement receptor family (Krych *et al.,* 1992), the gene coding for C3AR1 (complement component 3a receptor 1) is increased, but the gene coding for C1QR1 (complement component 1, q subcomponent, receptor 1) is decreased.

In short, we investigated VCA-induced alterations in the transcriptional profiles of human T lymphocytes and analyzed the differentially regulated genes using bioinformatics tools. This report aims at providing the mistletoe research community with a robust data base on which further studies could be built. To our knowledge, this is the first report on the effect of (European or Korean) mistletoe lectin on naïve human T-lymphocytes.

ACKNOWLEDGMENTS

This work was supported by a special research grant from Seoul Women's University in the program year of 2010.

REFERENCES

Alizadeh, A. A., Eisen, M. B., Davis, R. E., Ma, C., Lossos, I. S., Rosenwald, A., Boldrick, J. C., Sabet, H., Tran, T., Yu, X., Powell, J. I., Yang, L., Marti, G. E., Moore, T., Houdson, J. J., Lu, L., Lewis, D. B., Tibshirani, R., Sherlock, G., Chan, W. C., Greiner, T. C., Weisenburger, D. D., Armitage, J. O.,

Warnke, R., Levy, R., Wilson, W., Grever, M. R., Byrd, J. C., Botstein, D., Brown, P. O. and Staudt, L. M. (2000). Distinct types of diffuse large B-cell-lymphoma identified by gene expression profiling. *Nature* **403**, 503-511.

Barlow, B. A. (1983). Biogeography of loranthaceae and viscaceae. In *The Biology of Mistletoes* (A. Keast, Ed.), pp. 19-46. Academic Press, Paris, San Diego, San Francisco, Sao Paulo, Tokyo, Toronto.

Beuth, J., Ko, H., Tunggal, L., Buss, G., Jeljaszewiez, J., Steuer, M. K. and Pulverer, G. (1994). Immunaktive wirkung von mistellektin-1 in abhängigkeit von der dosierung. *Arzneimittelforschung* **44**, 1255-1258.

Büssing, A. (1997). Mistletoe therapy and immunological research. *Anticancer Drugs* **8(Suppl 1)**, S65.

Büssing, A. (2000). Biological and pharmacological properties of *Viscum album* L. In *Mistletoe: The Genus Viscum* (A. Büssing, Ed.), pp. 123-182. Harwood Academic Publishers, Amsterdam, The Netherlands.

Frantz, H. (1989). Viscaceae lectins. In *Advances in Lectin Research* (H. Frantz, Ed.), Vol. 2, pp. 28-59. VEB Verlag Volk und Gesundheit, Berlin.

Gleichmann, E., Kimber, I. and Purchase, I. F. (1989). Immunotoxicology: suppressive and stimulatory effects of drugs and environmental chemicals on the immune system. A discussion. *Arch. Toxicol.* **63**, 257-273.

Hajto, T., Hostanska, K., Frei, K., Rordorf, C. and Gabius, H. J. (1990). Increased secretion of tumor necrosis factor α, interleukin 1, and interleukin 6 by human mononuclear cells exposed to β-galactoside-specific lectin from clinically applied mistletoe extract. *Cancer Res.* **50**, 3322-3326.

Heyman, B. (1996). Complement and Fc-receptors in regulation of the antibody response. *Immunol. Lett.* **54**, 195-199.

Homey, B., Muller, A. and Zlotnik, A. (2002). Chemokines: agents for the immunotherapy of cancer? *Nat. Rev. Immunol.* **2**, 175-184.

Hostanska, K., Hajto, T., Spagnoli, G. C., Fischer, J., Lentzen, H. and Hermann, R. (1995). A plant lectin derived from *Viscum album* induces cytokine gene expression and protein production in cultures of human peripheral blood mononuclear cells. *Nat. Immun.* **14**, 295-304.

Hostanska, K., Hajto, T., Weber, K., Fischer, J., Lentzen, H., Sutterlin, B. and Saller, R. (1996). A natural immunity-activating plant lectin, *Viscum album* agglutinin-1, induces apoptosis in human lymphocytes, monocytes, monocytic THP-1 cells, and murine thymocytes. *Nat. Immun.* **15**, 295-311.

Joshi, S. S., Komanduri, K. C., Gabius, S. and Gabius, H. J. (1991). Immunotherapeutic effects of purified mistletoe lectin (ML-1) on murine large cell lymphoma. In *Lectins and Cancer* (H. J. Gabius and S. Gabius, Eds.), pp. 207-216. Springer Verlag, Heidelberg.

Krych, M., Atkinson, J. P. and Holers, V. M. (1992). Complement receptors. *Curr. Opin. Immunol.* **4**, 8-13.

Lavastre, V., Pelletier, M., Saller, R., Hostanska, K. and Girard, D. (2002). Mechanisms involved in spontaneous and *Viscum album* agglutinin-I-induced human neutrophil apoptosis: *Viscum album* agglutinin-I accelerates the loss of anti-apoptotic Mcl-1 expression and the degradation of cytoskeletal paxillin and vimentin proteins *via* caspases. *J. Immunol.* **168**, 1419-1427.

Li, S. Z. (1593). Material Medica Principles: describes 1,892 kinds of medical material in 1578 AD. In *Compendium of Materia Medica* (Bencao Ganmu), Vol. 1-52, pp. 145-168, People's Medical Publishing House, Beijing.

Lyu, S. Y., Choi, S. H. and Park, W. B. (2002). Korean mistletoe lectin-induced apoptosis in hepatocarcinoma cells is associated with inhibition of telomerase via mitochondrial controlled pathway independent of p53. *Arch. Pharm. Res.* **25**, 93-101.

Lyu, S. Y., Park, S. M., Choung, B. Y. and Park, W. B. (2000). Comparative study of Korean (*Viscum album* var. *coloratum*) and European mistletoes (*Viscum album*). *Arch. Pharm. Res.* **23**, 592-598.

Lyu, S. Y. and Park, W. B. (2007). Effects of Korean mistletoe lectin (*Viscum album coloratum*) on proliferation and cytokine expresssion in human peripharal blood mononuclear cells and T-lymphocytes. *Arch. Pharm. Res.* **30**, 1252-1264.

Lyu, S. Y., Park, W. B., Choi, K. H. and Kim, W. H. (2001). Involvement of caspase-3 in apoptosis induced by *Viscum album* var. *coloratum* agglutinin in HL-60 cells. *Biosci. Biotechnol. Biochem.* **65**, 534-541.

Männel, D. N., Becker, H., Gundt, A., Kist, A. and Franz, H. (1991). Induction of tumor necrosis factor expressed by a lectin from *Viscum album. Cancer Immunol. Immunother.* **33**, 177-182.

Möckel, B., Schwarz, T., Zinke, H., Eck, J., Langer, M. and Lentzen, H. (1997). Effects of mistletoe lectin I on human blood cell lines and peripheral blood cells. Cytotoxicity, apoptosis, and induction of cytokines. *Arzneimittelforschung* **47**, 1145-1151.

Pae, H. O., Oh, G. S., Kim, N. Y., Shin, M. K., Lee, H. S., Yun, Y. G., Oh, H., Kim, Y. M. and Chung, H. T. (2001). Roles of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase in apoptosis of human monoblastic leukemia U937 cells by lectin-II isolated from Korean mistletoe. *In Vitr. Mol. Toxicol.* **14**, 99-106.

Park, J. H., Hyun, C. K. and Shin, H. K. (1998a). Cytotoxicity of heat-treated Korean mistletoe. *Cancer Lett.* **126**, 43-48.

Park, W. B., Han, S. K., Lee, M. H. and Han, K. H. (1997). Isolation and characterizaton of lectins from stem and leaves of Korean mistletoe (*Viscum album* L. var. *coloratum*) by affinity chromatography. *Arch. Pharm. Res.* **20**, 306-312.

Park, W. B., Ju, Y. J. and Han, S. K. (1998b). Isolation and characterization of beta-galactoside specific lectin from Korean mistletoe (*Viscum album* var. *coloratum*) with lactose-BSA-sepharose 4B and changes of lectin conformation. *Arch. Pharm. Res.* **21**, 429-435.

Park, W. B., Lyu, S. Y., Kim, J. H., Choi, S. H., Chung, H. K., Ahn, S. H., Hong, S. Y., Yoon, T. J. and Choi, M. J. (2001). Inhibition of tumor growth and metastasis by Korean mistletoe lectin is associated with apoptosis and antiangiogenesis. *Cancer Biother. Radiopharm.* **16**, 439-447.

Reilly, M. P. and McKenzie, S. E. (2002). Mechanisms of action of IVIg: physiology of Fc receptors. *Vox Sang.* **83(Supple 1)**, 57-63.

Riberéau-Gayon, G., Dumont, S., Muller, C., Jung, M. L., Poindron, P. and Anton, R. (1996). Mistletoe lectins I, II and III induce the production of cytokines by cultured human monocytes. *Cancer Lett.* **109**, 33-38.

Riberéau-Gayon, G., Jung, M. L., Frantz, M. and Anton, R.

(1997). Modulation of cytotoxicity and enhancement of cytokine release induced by *Viscum album* L. extracts or mistletoe lectins. *Anticancer Drugs* **8**, S3-S8.

Richmond, A. (2002). Nf-kappa B, chemokine gene transcription and tumour growth. *Nat. Rev. Immunol.* **2**, 664-674.

Rossi, D. and Zlotnik, A. (2000). The biology of chemokines and their receptors. *Annu. Rev. Immunol.* **18**, 217-242.

Savoie, A., Lavastre, V., Pelletier, M., Hajto, T., Hostanska, K. and Girard, D. (2000). Activation of human neutrophils by the plant lectin *Viscum album* agglutinin-I: modulation of de novo protein synthesis and evidence that caspases are involved in induction of apoptosis. *J. Leukoc. Biol.* **68**, 845-853.

Schultze, J. L., Stettin, A. and Berg, P. A. (1991). Demonstration of specifically sensitized lymphocytes in patients treated with an aqueous mistletoe extract (*Viscum album* L.). *Klinische Wochenschrift* **69**, 397-403.

'Stvrtinová, V., Jakubovský, J. and Hulín, I. (1995). Pathophysiology. Principles of diseases. Computing Centre, Slovak Academy of Sciences, Slovakia Online.

Urech, K., Scher, J. M., Hostanska, K. and Becker, H. (2005). Apoptosis inducing activity of viscin, a lipophilic extract from *Viscum album* L. *J. Pharm. Pharmacol.* **57**, 101-109.

Yoon, T. J., Yoo, Y. C., Kang, T. B., Her, E., Kim, S. H., Kim, K., Azuma, I. and Kim, J. B. (2001). Cellular and humoral adjuvant activity of lectins isolated from Korean mistletoe (*Viscum album coloratum*). *Int. Immunol.* **1**, 881-889.

Yoon, T. J., Yoo, Y. C., Kang, T. B., Shimazaki, K., Song, S. K., Lee, K. H., Kim, S. H., Park, C. H., Azuma, I. and Kim, J. B. (1999). Lectins isolated from Korean mistletoe (*Viscum album coloratum*) induce apoptosis in tumor cells. *Cancer Lett.* **136**, 33-40.

Yoon, T. J., Yoo, Y. C., Kang, T. B., Song, S. K., Lee, K. B., Her, E., Song, K. S. and Kim, J. B. (2003). Antitumor activity of the Korean mistletoe lectin is attributed to activation of macrophages and NK cells. *Arch. Pharm. Res.* **26**, 861-867.